



## Replacement Sheet

**MKK4** (cDNA accession number: AB015315)

*Wild-type cDNA sequence from **start** to **end** codons  
(1101 base pairs including the end TAG):*

**atg**agaccgattcaatcgccctccaggagtttccgttccggtgaaaagccgtccccgctcg  
ccgtcctgatcttaccttaccgcttccctcaacgcgatgtttctctcgtgtacctcttc  
ctctcccacctacttccggtgggttccggtggctctagtggatctgcgccgtcttctggg  
ggttcggcgtcttcaacgaacactaacagctccatagaagcgaagaactattcggattt  
agtgagaggtaaccgtatcggaagcggagcaggtggaacggtatacaaagtgattcacc  
gtccgagttctcgtctatatgcacttaaggatgatacggtaaccacgaggagactgtg  
agacgtcagatctgtagagagatcgagattttacgagatgtgaatcatccaaacgttgt  
gaaatgtcacgagatgtttgatcagaacggtgagatccaggttttgcttgagtttatgg  
ataaagggttctttagaagggtgctcatgtgtggaaagagcaacaattagctgatctatct  
cgtcagattcttagtggtttagcttatctccatagccgtcacatagttcatcgtgatat  
caaaccatcgaatcttttgataaactctgctaaaaacgttaagattgctgattttggag  
ttagtaggatcttgggtcagactatggatccgtgtaattcctctgttggaaccattgct  
tatatgagtcctgagaggattaacactgatttgaatcagggaaagtatgatggttatgc  
tggagatatttggagcttaggtgttagcattttggagttttacttggggaggtttcctt  
tcctgtgtagtagacaagggtgattgggctagtcttatgtgtgccattttgtatgtctcag  
cctccagaagctccagcgactgcgtcgccggagtttcggcattttatctcgtgttgctt  
gcagagagaaccggggaaaaggaggagtgtatgcagctattgcagcatcctttcatat  
taagagcaagtccgagccagaacaggtctcctcagaatctacatcaactcttgccctct  
cctcgtcctctgtcctcgtcttcttctccaaccacatag (SEQ ID NO.:3)

*Wild-type protein sequence (366 aminoacids):*

MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGSAPSSG  
GSASSTNTNSSIEAKNYSIDLVRGNRIGSGAGGTVYKVIHRPSSRLYALKVIYGNHEETV  
RRQICREIEILRDVNHHPNVVKCHEMFDQNGEIQVLLEFMDKGSLEGAHVWKEQQLADLS  
RQILSGLAYLHSRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQIMDPCNSISVGTIA  
YMSPERINTDLNQGYDGYAGDIWSLGVSI LEFYLG RFPFPVSRQGDWASLMCAICMSQ  
PPEAPATASPEFRHFISCLQREPGKRRSAMQLLQHPFILRASPSQNRSPQNLHQLLPP  
PRPLSSSSSPTT (SEQ ID NO.:4)

**FIGURE 4**

# Replacement Sheet

## **Mutations rendering MKK4 constitutively active:**

- modify Threonine (T) 224 to Aspartic Acid (D) by changing codon ACT into GAT  
- modify Serine (S) 230 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCTCAGgaTATGGATCCGTGTAATgaATCTGTTGGAAC  
TCCAACAGATtGATTACACGGATCCATAtcCTGAGCCAAG (SEQ ID NO.3)

So the sequences after mutations are:

**MKK4act mutant sequence from start to end codons  
(1101 base pairs including the end TAG):**

atgagaccgattcaatcgctccaggagtttccggtccggtgaaaagccggtcccgctcgccgtcctgatct  
taccttacgcttccctcaacgcatgtttctctcgctgtacctcttccctctccacactacttccggtgggt  
ccggtggctctagtggatctgcgcgctcttctggtgggtccggtcttcaacgaacactaacagctccata  
gaagcgaagaactattcggatttagtgagaggttaaccgtatcggaagcggagcaggtggaacggtatacaa  
agtgattcacggtccgagttctcgtctatatgcacttaagggtgatatacggtaaccacgaggagactgtga  
gacgtcagatctgtagagagatcgagattttacgagatgtgaatcatccaaacggttgtaaagtgtcacgag  
atgtttgatcagaacggtgagatccaggttttgcttgagtttatggataaagggttctttagaagggtgctca  
tgtgtggaagagcaacaattagctgatctatctcgtcagattcttagtggttttagcttatctccatagcc  
gtcacatagttcatcgtgatatacaaccatcgaatcttttgataaactctgctaaaaacggttaagattgct  
gattttggagtttagtaggatcttgggtcaggatgatggatccggtgtaatgaatctgttggaaccattgctta  
tatgagtcctgagaggattaacactgatttgaatcagggaaggtatgatggttatgctggagatatttggga  
gcttaggtgttagcatttttgaggttttacttggggagggtttcccttccctgtgagtagacaagggtgattgg  
gctagtcttatgtgtgccatttgtatgtctcagcctccagaagctccagcgactgcgtcgccggagtttcg  
gcattttatctcgtgttgcttgagagagaacgggggaaaggaggagtgctatgcagctattgcagcatc  
ctttcatattaagagcaagtcgagccagaacaggtctcctcagaatctacatcaactcttgccctcctct  
cgtcctctgtcctcgtcttcttctccaaccacatag (SEQ ID NO.:6)

**MKK4act mutant protein sequence (366 aminoacids):**

MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGSAPSSSGGSASSTNTNSSI  
EAKNYSIDLVRGNRIGSGAGGTVYKVIHRPSSRLYALKVIYGNHEETVRRQICREIEILRDVNHNPVVKCHE  
MFDQNGEIQVLLFMDKGSLEGAHVWKEQQLADLSRQILSGLAYLHSRHIVHRDIKPSNLLINSKENVKIA  
DFGVSRILAQMDPCNESVGTIAYMSPERINTDLNQKYGAGDIWSLGVSI LEFYLGFRFPFPVSRQGDW  
ASLMCAICMSQPPEAPATASPEFRHFISCCLOREP GKRRSAMQLLQHPFILRASPSQNRSPQNLHQLPPP  
RPLSSSSSPTT (SEQ ID NO.:7)

**FIGURE 5**

# Replacement Sheet

**MKK5** (cDNA accession number: AB015316)

*Wild-type cDNA sequence from **start** to **end** codons  
(1047 base pairs including the end TAG):*

**atg**aaaccgattcaatctccttctggagtagcttcacctatgaagaaccgtttacgcaa  
acgtcctgacctaagcttaccactcccacaccgcgacgtcgctctcgccgtacctctcc  
ctctcccacctccttcttctcttccatccgctccggcgtcttccctccgcgatctcaacc  
aacatctccgcccgtataaagcttatccgagctagaacgagtgaaaccgaatcggaagcgg  
agccggaggaaacggtttacaaagtaatccacactccgacgtcacgtcctttcgctctca  
aagtgatttacggaaccacgaagataccgtgagacgtcagatctgtagagagatcgag  
atcttaagaagtgttgatcatccaaacgttgtgaaatgtcacgatatgtttgatcataa  
cggtagatccagggttttgcttgagtttatggatcaaggatctcttgaggagctcata  
tatggcaagaacaggaattagctgatctctctcgtcagattcttagtgattagcttat  
cttcacgtcgtcatatcgttcatcgatataaaaccttcgaatctccttataaaactc  
agctaaaaatgtgaaaattgctgattttgggtgtgagtaggatcttggcacaaa**aat**gg  
atccttgtaatt**cat**ctgttggtactattgcttatatgagtcctgagaggattaatact  
gatttgaatcatggtcgttacgatggttatgctggagatgtttggagtttaggtgttag  
tatcttgaggttttacttggggagggtttccttttgctgtgagtagacaagggtgattggg  
ctagtcttatgtgtgctatttgtatgtctcagccacctgaagctccggctacggcgtct  
caggagtttcgtcactttgtttcttggtgtttacagagtgatcctcctaagagatggtc  
agctcaacagcttttgcagcatcctttcatacttaaagctaccggtggctctaattctcc  
gtcaaattgttgccgccgcctcgtcctcttctcttgcctct**tag** (SEQ ID NO.:8)

*Wild-type protein sequence (348 aminoacids):*

MKPIQSPSGVASPMKNRLRKRPDL~~SL~~PLPHRDVALAVPLPLPPPSSSSSSAPASSSAIST  
NISA~~AKSL~~SELERVNRIGSGAGGT~~VY~~KVIHTPTSRPFALKVIYGNHEDTVRRQICREIE  
ILRSVDHPNVVKCHDMFDHNGEIQVLLEFMDQGSLEGAHIWQEQELADLSRQILSGLAY  
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQ**IM**DPCN**S**SVGTIAYMSPERINT  
DLNHGRYDGYAGDVWSLGV~~S~~ILEFYLG~~R~~FPFAVSRQGDWASLMCAICMSQPPEAPATAS  
QEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATGGPNLRQMLPPPRPLPSAS (SEQ  
ID NO.:9)

FIGURE 6

# Replacement Sheet

## **Mutations rendering MKK5 constitutively active:**

- modify Threonine (T) 215 to Glutamic Acid (E) by changing codon ACA into GAA  
- modify Serine (S) 221 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCACAgaAATGGATCCTTGTAATgaATCTGTTGGT  
ACCAACAGATtcATTACAAGGATCCATtctTTGTGCCAAG (SEQ ID NO.:10)

So the sequences after mutations are:

**MKK5act mutant cDNA sequence from start to end codons (1047 base pairs including the end TAG):**

atgaaaccgattcaatctccttctggagtagcttcacctatgaagaaccgtttacgcaa  
acgtcctgacctaagcttaccactcccacaccgcgacgtcgctctcgccgtacctctcc  
ctctcccacctccttcttctcctcttcacccgctccggcgtcttctccgcgatctcaacc  
aacatctccgccgctaaaagcttatccgagctagaacgagtgaaaccgaatcggaagcgg  
agccggaggaacggtttacaaagtaatccacactccgacgtcacgtcctttcgctctca  
aagtgatttacggaaccacgaagataccgtgagacgtcagatctgtagagagatcgag  
atcttaagaagtgttgatcatccaaacggttgtaaagtgtcacgatatgtttgatcataa  
cggtgagatccaggttttgcttgagtttatggatcaaggatctcttgaggagctcata  
tatggcaagaacaggaattagctgatctctctcgtcagattcttagtgagtagcttat  
cttcacgtcgatcatatcggttcacgtgatataaaaccttcgaatctccttataaaactc  
agctaaaaatgtgaaaattgctgattttgggtgtgagtaggatcttggcacaagaatgg  
atccttgtaatgaatctgttggtactattgcttatatgagtcctgagaggattaatact  
gatttgaatcatggtcgttacgatggttatgctggagatgtttggagtttaggtgttag  
tatcttgaggttttacttggggaggtttccttttgctgtgagtagacaagggtgattggg  
ctagtcttatgtgtgctatttgtatgtctcagccacctgaagctccggctacggcgtct  
caggagtttcgtcactttgtttcttggtgtttacagagtgatcctcctaagagatggtc  
agctcaacagcttttgcagcatcctttcatacttaaagctaccggtggtcctaatactcc  
gtcaaatgttgccgccgcctcgtcctcttctctcttgcctcttag (SEQ ID  
NO.:11)

**MKK5act mutant protein sequence (348 aminoacids):**

MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPSSSSSAPASSSAIST  
NISAAKSLSELERVNRIGSGAGGTVYKVIHTPTSRPFALKVIYGNHEDTVRRQICREIE  
ILRSVDHPNVVKCHDMFDHNGEIQVLLFMDQGSLEGAIHWQEQELADLSRQILSGLAY  
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQEMDPCNE<sup>215</sup>SVGTIAYMSPERINT  
DLNHGRYDGYAGDVWSLGVSILEFYLGFRPF<sup>221</sup>FAVSRQGDWASLMCAICMSQPPEAPATAS  
QEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATGGPNLRQMLPPPRPLPSAS (SEQ  
ID NO.:12)

FIGURE 7

# Replacement Sheet

## Alignment of MKK4 and MKK5 wild-type:

MKK4 MRPIQSPPGVSVPVKSRPRRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGG  
MKK5 MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPS-----

MKK4 SSGSAPSSGGSASSTNTNSSIEAKNYS DLVRGNRIGSGAGGT VYKVIHRP  
MKK5 SSSSAPASS-SAISTNIS---AAKSLSELERVNRIGSGAGGT VYKVIHTP

MKK4 SSRLYALKVIYGNHEETVRRQICREIEILRDVNHPNVVKCHEMFDQNGEI  
MKK5 TSRPFALKVIYGNHEDTVRRQICREIEILRSVDHPNVVKCHDMFDHNGEI

MKK4 QVLLFMDKGSLEGAHVWKEQQ LADLSRQILSGLAYLHSRHIVHRDIKPS  
MKK5 QVLLFMDQGSLEGAHIWQEQLADLSRQILSGLAYLHRRHIVHRDIKPS

MKK4 NLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIAYMSPERINTDLNQG  
MKK5 NLLINSAKNVKIADFGVSRILAQTMDPCNSSVGTIAYMSPERINTDLNHG

MKK4 KYDGYAGDIWSLGVSI LEFYLG RFPFPVSRQGDWASLMCAICMSQPPEAP  
MKK5 RYDGYAGDVWSLGVSI LEFYLG RFPFAVSRQGDWASLMCAICMSQPPEAP

MKK4 ATASPEFRHFISCC LQREPGKRRS AMQLLQHPFILRASPSQNRSPQNLHQ  
MKK5 ATASQEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATG---GP-NLRQ

MKK4 LLPPPRPLSSSSSPTT (SEQ ID NO.:13)  
MKK5 MLPPPRPLPSAS---- (SEQ ID NO.:14)

FIGURE 8